Research Journal of Medical Sciences 2 (1): 7-12, 2008
ISSN: 1815-9346

Re-Sterilization of Surgical Polypropylene Meshes: Should it be Done?

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Abstract: In times of reduced financial margins, resterialization of surgical meshes is a possibility to economise their use. To test whether re-sterilization of polypropylene meshes (initially gas-sterilized) by steam autoclaving is advisable we analyzed resterilized specimens on structural alterations and the release of cytotoxic substances. Gas-sterilized (non-resterilized) and resterilized (121°C, 20 min) nonabsorbable PROLENE (Ethicon, Germany) meshes (1 cm^2) incubated 48 h under cell culture conditions were imaged using scanning electron microscopy. Potential release of substances from original and resterilized meshes was analyzed on lyophilisates using mass spectrometry. Toxicity of lyophilisates was tested on cultures of primary fibroblasts by estimation of proliferation and apoptotic index. Original meshes showed a smooth surface. Filaments of resterilized meshes exhibited tears and crevices of variable depth. After incubation under cell culture conditions, filaments of non-resterilized and especially resterilized meshes revealed tears and crevices. Dots of variable size have formed on the surface. Mass spectrograms of resterilized meshes demonstrated release of polymer molecules with a mass difference of 44 amu. Finally, apoptotic index of fibroblasts incubated with lyophilisates of resterilized meshes was significantly elevated. Re-sterilization of polypropylene meshes leads to distinct material defects on filament's surface and a release of substances which causes apoptosis in human fibroblasts. For this reason, re-sterilization of polypropylene meshes should be avoided.

Key words: Polypropylene meshes, re-sterilization, proliferation, apoptosis

INTRODUCTION

Repair of inguinal and incisional hernias with synthetic meshes is a widely accepted method for a long time and has significantly reduced the rate of postoperative recurrences in comparison to Mayo procedure (Liakakos *et al*., 1994). But in our times improvement of surgical results alone is of limited relevance for new treatment options, the price of the implant is another important aspect. From this point of view, re-sterilization of surgical meshes could be an option to reduce costs. This study was conducted to evaluate meshes and the effects of re-sterilization in an *in vitro* model.

Besides mechanical closure of the hernial orifice mesh implantation leads to the induction of scar tissue and therefore is an important factor for stabilization and strengthening of the abdominal wall (Klinge *et al*., 1999; Klosterhalfen *et al*., 1997; Schumpelick *et al*., 1999). Common histological examinations demonstrated a more or less acute and chronic inflammatory response in the area of implanted meshes followed by tissue fibrosis, depending on the structure of the incorporated material (Schumpelick *et al*., 1999; Anderson, 1988; Klosterhalfen *et al*., 2000; Rosch *et al*., 2003).

Despite a great variety of materials polypropylene meshes are frequently applied in hernia repair since its introduction by Usher (1959). There exist many short and long term studies about the biocompatibility of polypropylene meshes and its modifications, in particular regarding the inflammatory response in human and animal tissues (Klinge *et al*., 1999; Klosterhalfen *et al*., 2000; Di Vita *et al*., 2000; Klosterhalfen *et al*., 1998; Rosch *et al*., 2003; Scheidbach *et al*., 2004). In contrast, examinations regarding the interaction between cells and meshes on molecular level are comparatively rare (Broll *et al*., 2002; Duchrow *et al*., 2002; Junge *et al*., 2002, 2003; Rosch *et al*., 2002; Schachtrupp *et al*., 2003).

Polypropylene meshes (e.g. Prolene®, Ethicon, Norderstedt, Germany) are initially sterilized by ethylene

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oxide and re-sterilization is not recommended by the producer. However, product information states that „...testing has demonstrated that reprocessing of unused Prolene mesh which has been removed from the package will not be adversely affected when exposed not more than one time to conventional steam autoclave conditions of 250°F (121°C) for 20 min. (Ethicon Inc. product information 389392. R02: http://www.jnjgateway.com/public/USENG/6944PROLENE_Polypropylene_Mesh.pdf).

The potential economic benefit can be easily demonstrated in a simple calculation: The suggested list price for a 10×15 cm Prolene mesh (Ethicon, Norderstedt, Germany) amounts 64,20 Euro (€). By cutting a 30×30 cm large mesh (price: 178,20 €) into 6 parts of 10×15 cm and resterialization of these parts 207,00 € will be saved (The cost of resterialization must be deducted). Based on this information it is a common practice in surgical departments to trim large polypropylene meshes required for hernia repair and re-sterilize the parts of the mesh for further use.

Recently we were able to demonstrate in an *in vitro* model the influence of polypropylene meshes on cell proliferation and apoptosis in human fibroblast cultures. We found that proliferative activity of cells decreased slightly from 85-75% after 48 h of incubation with meshes, whereas apoptotic index increased from 2-19% (Duchrow *et al*., 2002). When using resterilized meshes, the proliferation index decreased significantly from 85- 42% and apoptotic index increased significantly from 2- 48% (Broll *et al*., 2002).

Encouraged by these results, the aim of the present study was to assess the effects of re-sterilization on the structural integrity of polypropylene meshes and subsequent influences on cytotoxicity after exposure to human fibroblast cultures. Moreover, a mass spectrometry was carried out to further characterize the substances released by the re-sterilization process.

MATERIALS AND METHODS

Meshes: Sterile nonabsorbable polypropylene meshes (Prolene®, Ethicon, Norderstedt, Germany) were cut into pieces of 1 cm^2 in size under sterile conditions. One half of the specimens were resterilized in a steam autoclave (Webeco, Bad Schwartau, Germany) according to the manufacturer's recommendations (121°C, 20 min).

Cells: Primary cells of Human Fibroblasts (HFIB, Cell-lining, Berlin, Germany) were cultured under standard conditions in RPMI-1640 medium (Gibco BRL, Berlin, Germany) supplemented with 10% fetal calf serum, glutamine, HEPES buffer (Gibco BRL, Berlin, Germany) and antibiotics until a monolayer was seen. Cells were harvested and seeded into six-well culture plates in a concentration of 3×10^4 cells. The viability of cells was tested by means of trypan blue staining and was constantly more than 98%.

Scanning electron microscopy: For scanning electron microscopy specimens were divided into three groups. Group 1: Non-resterilized and resterilized meshes processed as described above were examined directly by scanning electron microscopy. Group 2: Non-resterilized and resterilized meshes were incubated in six-well culture plates with cell culture medium at 37°C for 48 h. Group 3: Non-resterilized and resterilized meshes were incubated in six-well culture plates with fibroblasts and cell culture medium at 37°C for 48 h.

All specimens were dried in a critical point dryer (E 3000, Quorum Technologies, Newhaven, England) and flatly mounted on aluminium plates. After sputter-coating with platinum-palladium examinations were carried out with a scanning electron microscope (SEM 505, Philips, Eindhoven, The Netherlands) operated at 10-15 kV and findings were documented on Agfapan APX 100 films (Agfa, Cologne, Germany). Each experiment was carried out four times.

Mass spectrometry: To verify whether the apoptotic effects on cultured human fibroblast described previously (Broll *et al*., 2002; Duchrow *et al*., 2002) are caused by cytotoxic substances released from resterilized meshes, extracts of soluble components derived from mesh specimens were prepared and characterized by mass spectrometry.

Non-resterilized and resterilized meshes were incubated at 37°C for 48 h in 1 mL aqua dest. in sterile glass containers. A sterile glass container only filled with 1 ml aqua dest. served as a control. After removal of the meshes the remaining solutions were concentrated by lyophilisation using the laboratory freeze dryer alpha 1-2 (Christ, Osterode, Germany). The lyophilisates were dissolved in 50 μL aqua dest. and analysed by Electrospray Ionisation (ESI) Fourier-transform mass spectrometry, ESI FT-MS (Apex II, Bruker Daltonics, Billerica, MA, USA). After addition of the spray solution (a 50:50:0.03 (v/v/v) mixture of 2-propanol, water, 30mM ammonium acetate adjusted with acetic acid to pH 4.5) the samples were sprayed at a flow rate of 2 μL min^{-1} and were analysed in the positive ion mode. Each experiment was carried out four times.

Incubation of fibroblasts with lyophilisates and determination of apoptotic index: Human fibroblasts were seeded into six-well culture plates in a concentration of

3×10^4 cells and 50 µL of lyophilisates obtained from non-resterilized and resterilized meshes were added. 50 µL aqua dest. served as control. After 48 h fibroblasts were harvested and apoptotic indices were assessed as described previously (13,14). Briefly, fibroblasts were immunocytochemically stained using the apoptosis detection kit (Pharmingen, Heidelberg, Germany; 5 µL FITC-conjugated Annexin-V; 15 min incubation at room temperature) and apoptotic indices determined by flow cytometry (FACScan, Becton Dickinson, Heidelberg, Germany) using the software CellQuest, version 3.2 (Becton Dickinson, Heidelberg, Germany). Unstained fibroblasts served as negative controls. Experiments were repeated four times and mean value calculated.

Statistical analysis: Results were expressed as mean values ± standard deviation (SEM). Statistical analysis was performed using SPSS software program, Version 11.0 (SPSS Inc., Chicago, Ill., USA). Differences between groups were tested by the Mann-Whitney U-test with p values $\leq$ 0.05 considered to be significant.

This study has been conducted in the Surgical Research Laboratory, Surgical Clinic, University Hospital Schleswig-Holstein, Campus Luebeck in Germany between 2004 and 2005.

RESULTS

Scanning electron microscopy

Group 1 (non-resterilized/resterilized meshes): On gross overview filaments of non-resterilized meshes showed a rather smooth surface. Higher magnifications revealed that the filaments were characterized by small, flattened, homogeneously distributed and parallel orientated furrows running along the longitudinal filament axis (Fig. 1A, B). These features resemble the normal appearance of non-resterilized meshes with a completely preserved integrity of its filaments.

In contrast, resterilized meshes exhibited a structurally altered surface morphology. Frequently the filaments revealed tears and crevices of variable depth giving evidence that part of the material has been cracked or ripped off (Fig. 1C, D).

Groups 2 and 3 (non-resterilized/resterilized meshes after incubation in culture medium, respectively exposed to cultured fibroblasts): After contact to the culture medium both non-resterilized and resterilized mesh specimens revealed tears and crevices on the surface of filaments (Fig. 2A, B). In addition, black dots of variable sizes have formed on the surface most likely reflecting initial signs of superficial material disintegration (Fig. 2B, C). Similar findings have been observed in those specimens exposed to cultured fibroblasts. However, the observed structural alterations of the surface were more frequently distributed and pronounced in resterilized than in non-resterilized specimens.

Mass spectrometry: The results of the mass spectrometric analyses are exemplarily demonstrated in Fig. 3. The control with aqua dest (Fig. 3A) showed only a few peaks originating mainly from contamination with calibration

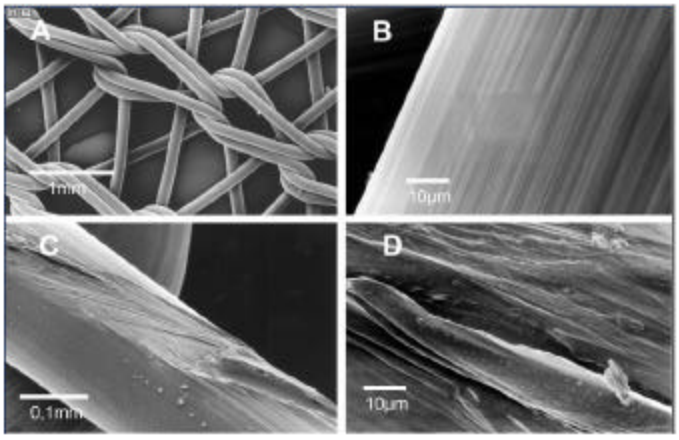

Fig. 1: Scanning electron micrographs of non-resterilized (A, B) and resterilized meshes (Prolene®) (C, D). Non-resterilized mesh specimen showing a smooth surface at overview magnification (A). At higher magnification the filaments are characterized by superficial parallel oriented furrows along the longitudinal filament axis (B). Resterilized mesh specimen with a structurally altered surface morphology (e.g. tears and crevices) of the filaments (C, D)

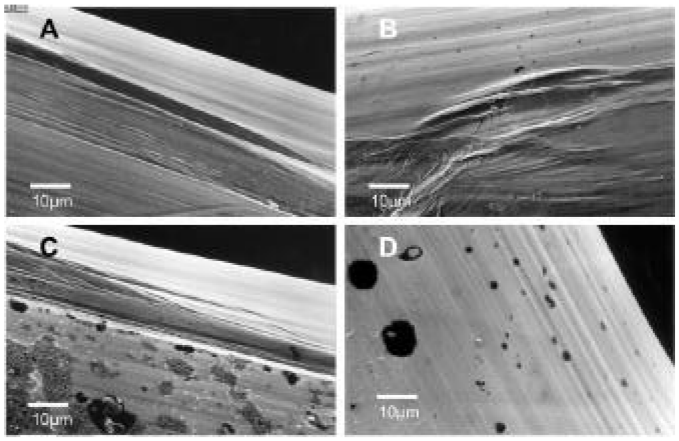

Fig. 2: Scanning electron micrographs of resterilized meshes (Prolene®) after incubation with culture medium (A, C) and cultured fibroblasts (B, D). After both procedures the filaments exhibited tears and crevices along their longitudinal axis (A, B) and additionally the formation of dot-like alterations as signs of superficial material disintegration (C, D)

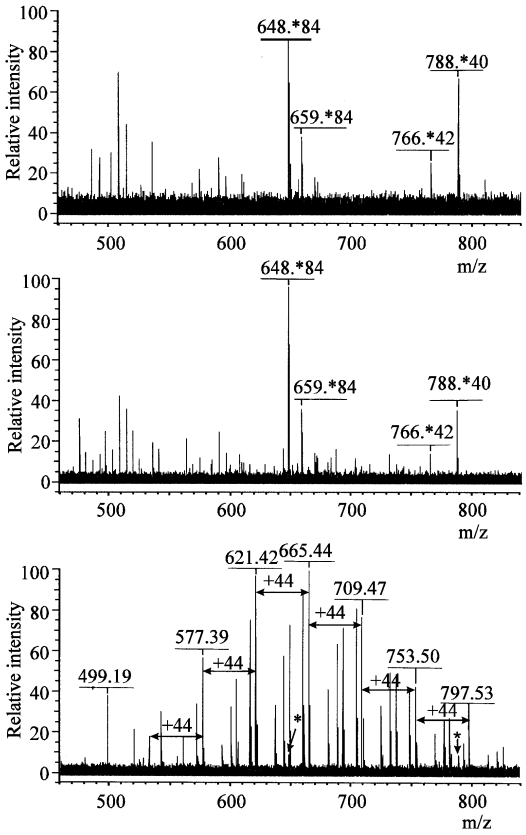

Fig. 3: ESI FT-MS mass spectra obtained in the positive ion mode of different lyophilisates. Control (aqua dest) (A); soluble products of a non-resterilized mesh specimen (B) and of a resterilized mesh specimen (C). The mass peaks labelled by (*) originate from calibration standards.

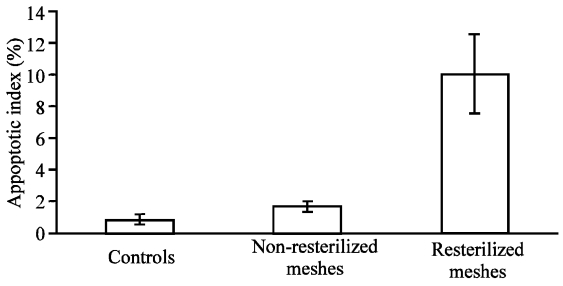

Fig. 4: Apoptotic index of fibroblasts after incubation with aqua dest (controls) with lyophilisates of non-resterilized and resterilized meshes

standards (labelled by *) The analysis of the samples obtained from the non-resterilized mesh specimens exhibited nearly the same pattern of mass peaks (Fig. 3B). In contrast, the mass spectrum of the lyophilisates obtained from resterilized meshes provided a complete different set of peaks in the mass range between m/z (mass/elementary charge) 500-800 (Fig. 3C) with constant mass differences of 44 amu Such pattern is indicative for polymers with different numbers of repeating units.

Apoptotic index of fibroblasts after incubation with lyophilisates: The apoptotic index as determined by flow cytometry of fibroblasts incubated with lyophilisates of resterilized meshes was 10%. In contrast, both fibroblasts incubated with lyophilisates of non-resterilized meshes and controls yielded an apoptotic index of 1.65 and 0.85 %, respectively. All these differences were significant ($p = 0.021$) at statistical analysis (Fig. 4).

DISCUSSION

Re-sterilization of meshes used for hernia repair can lead to a valuable amount of cost reduction in surgical departments with numerous hernia-operations. With the potentiality of this procedure given in the product informations of some producer this could be widely used to reduce the cost of tension free hernia repair compared to other suture techniques (e.g. Shouldice-repair, incisional hernia repair). By resterialization of e.g. Prolene mesh the price of a single 10×15 cm mesh can be reduced from 64,20-29,70€.

But there is a lack of information about the influence of re-sterilization on the mesh integrity and the effects of resterilized meshes on human tissue. So far, no examinations have been carried out to address these clinically relevant issues.

In a previous study, using an *in vitro* model we could demonstrate a significant increase of the apoptotic index of cultured human fibroblasts exposed for 48 h to resterilized meshes in contrast to non-resterilized meshes (Broll *et al*., 2002). Based on these observations the present study was aimed to characterize structural alterations of meshes subjected to re-sterilization and to clarify whether a toxic substance is released from mesh filaments during the process of re-sterilization.

To visualize structural changes of mesh filaments we used scanning electron microscopy allowing a detailed appraisal of the surface morphology. This method has been previously applied in a study by Klosterhalfen *et al*. (2000) for the assessment of meshes (Polypropylene, Polyester, PTFE) explanted 2 to 98 months after implantation due to infection or hernia recurrence. They found both a degradation and bacterial contamination of mesh filaments with and without clinical signs of infection.

In our study, resterilized meshes exhibited a structurally altered surface morphology of the filamentous compounds with tears and crevices of variable depth. In contrast, filaments of non-resterilized meshes were characterized by flattened, homogeneously distributed furrows along the longitudinal axis without any evidences of altered integrity. These findings clearly indicate that conventional re-sterilization procedures induce a considerable disintegration of the filamentous material of the meshes.

Moreover, when exposed to culture medium and cultured fibroblasts, both non-resterilized and resterilized meshes revealed similar defects. However, they were more pronounced in filaments previously subjected to re-sterilization. This observation supports our speculation that re-sterilization per se as well as long term contact to liquid and cellular material cause a remarkable alterations of the integrity of mesh filaments.

Obviously the material disintegration induced by re-sterilization is accompanied by the release of substances. Mass spectrometry (Zhang *et al*., 2004) demonstrated the release of polymer molecules with a mass difference of 44 amu most likely corresponding to Polyethylene Glycol (PEG) or Polyvinyl Alcohol (PVA). Both PEG and PVA are well known to be used as additives for the engineering of polypropylene during the production process. Lyophilisates from resterilized meshes significantly increased the apoptotic index of human fibroblast cultures in comparison to the control group and to lyophilisates obtained from non-resterilized meshes. If this adverse effect is caused by these polymeric substances identified by mass spectrometry or by other substances or small particles which were not detectable by mass spectrometry has to be confirmed.

CONCLUSION

The obtained data give, for the first time, evidence that re-sterilization of polypropylene meshes leads to a distinct loss of their surface integrity. Although the defects are not evident macroscopically, they can be readily identified by scanning electron microscopy. The substances released during the material disintegration of meshes exert toxic cellular effects *in vitro*, thereby confirming the findings from our previous study (Broll *et al*., 2002). Taken together, it is concluded from the data that re-sterilization of non-absorbable polypropylene meshes should be avoided due to the induced damage of filaments and subsequent cytotoxic effects.

Re-sterilization of surgical meshes isn´t an adequate option to reduce the costs of a tension free hernia repair according to these results.

ACKNOWLEDGEMENT

Thanks to Ms. A. Aumueller for technical assistance in cell culture experiments, Ms. C. Killaitis for statistical analysis and Ms. U. Windhoevel for critical reading of the manuscript.

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